

INSTRUCTION MANUAL

Model 810A

In-Vitro Test Apparatus for 310B Muscle Lever

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1.0 Introduction

The 810A in-vitro test apparatus for a 310B muscle lever system was designed to enable physiology researchers to easily test intact muscle tissue with an ASI model 310B muscle lever system. The 810A consists of a vertical mounting plate, legs, motor mount, tissue bath, vertical translation stage for the bath, lower muscle clamp, electrode holder and a vertical translation stage for the lower clamp. Also included are an oxygenating bubbler, tube clamp, motor cable clamp, screws to mount the motor to the motor mounting plate and metric and English Allen key sets. All parts are manufactured from corrosion resistant materials (anodized aluminum, stainless steel or Delrin). The vertical mounting plate is an extrusion with vertical Tee slots that allow additional equipment to be mounted on the plate. These Tee slots also allow all of the apparatus to be repositioned on the mounting plate by simply loosening a screw and sliding the apparatus to the new position.

The 810A provides flexibility to the researcher by including a vertical translation stage for the tissue bath that allows the bath to be easily raised or lowered for access to the tissue. The position of the bath with respect to the 310B lever arm and with respect to the lower tissue clamp can be adjusted with stops located on the vertical mounting plate.

The location of the lower tissue clamp can be adjusted through a wide range by repositioning the lower tissue clamp assembly on the vertical mounting plate. In addition a fine position adjustment is provided to allow the resting tension of resting length of the muscle tissue to be adjusted.

The position of the electrode clamp can also be adjusted over a wide vertical range by simply loosening the clamp screw and repositioning the electrode clamp anywhere along the length of the lower tissue clamp support tube.

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the shipping container. The 810A comes assembled but the glass tissue bath and bath accessories are packaged in a separate box contained within the main shipping container. Because it is constructed of glass, care must be taken when handling the tissue bath. Enclosed in a second box are the motor mount screws and sets of metric and English Allen keys. Remove all parts from the shipping container. Setup the main plate on a sturdy work surface (lab bench).

Caution: the 810A weighs about 80 lb so two people are required to lift it into place. See photo on next page for the correct setup. Also refer to the drawing AS810-A01 at the end of this manual for a diagram of the setup.

2.2 Attaching the 310B Motor

Before mounting the 310B motor to the 810A first attach the lever arm to the motor with the arm oriented horizontally and pointing to the left when viewing the motor from the shaft end.

Mount the 310B motor on the motor mount plate with the shaft of the motor pointing towards the right side of the apparatus (pointing towards the lower tissue clamp) and the lever arm pointing out from the 810A vertical plate. Remove the four M6 motor mount screws from the package and attach the motor to the plate using the metric Allen keys provided. Hang a wire or thread from the lever arm and then position the motor such that the thread lines up with the centre of the lower tissue clamp. The motor mount plate has slots in it to allow the position of the motor to be adjusted with respect to the lower tissue clamp. Once the motor is in the correct position tighten the motor mounting screws.

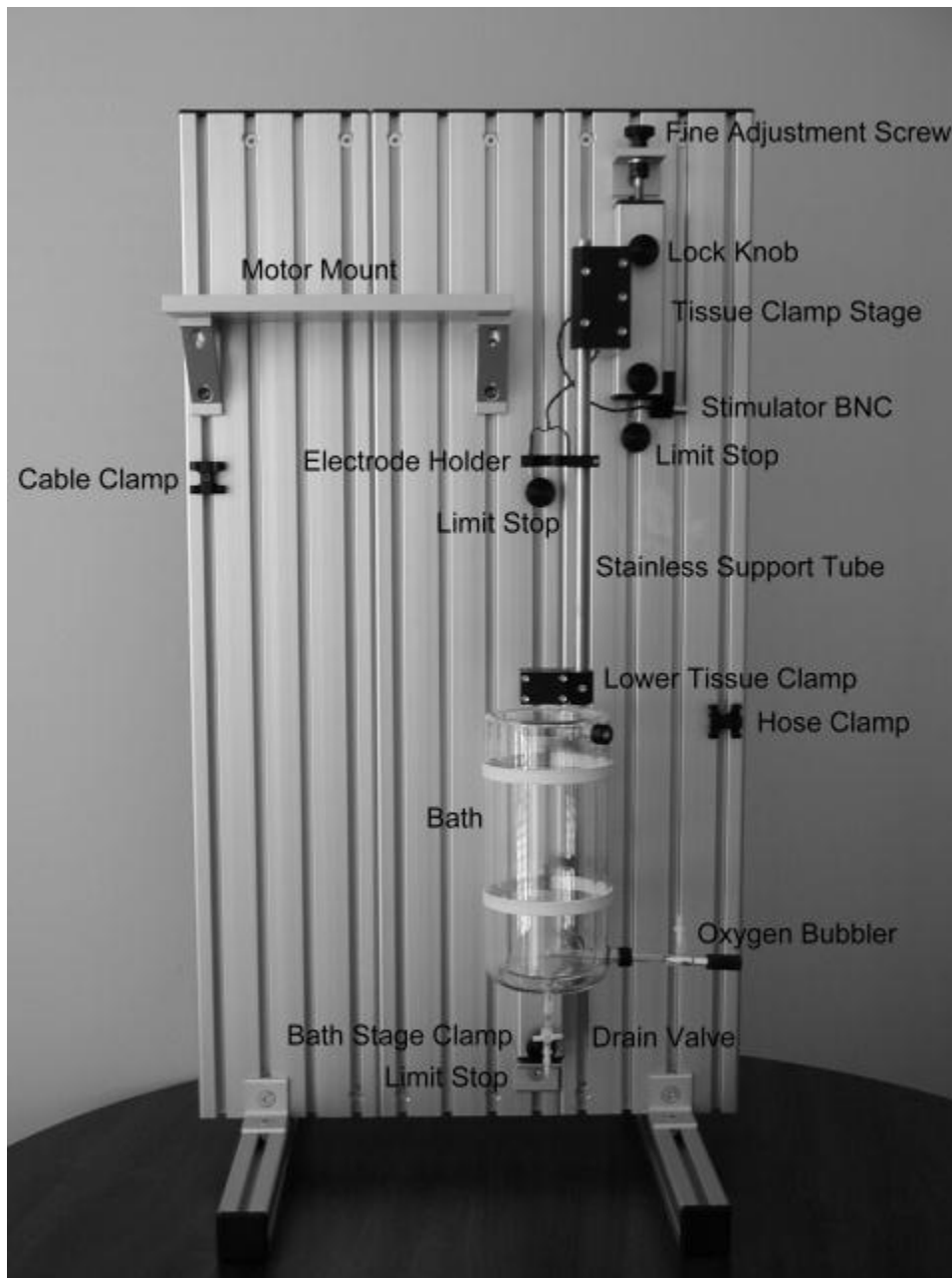
Use the black plastic clamp located on the left side of the vertical plate directly beneath the motor to clamp the motor cable to the 810A vertical plate. The clamp can be repositioned to any convenient location if so desired. The clamp can also be located on the back surface of the vertical plate if it is desired to keep the front side clear of wires.

2.3 Attaching the Tissue Bath

Unpack the Radnoti tissue bath and attach the bath to the 810A by inserting the bath clamp rods into the holes on the front of the bath vertical translation stage. Note: there are two screws on the right side of the bath vertical translation stage that must be loosened before the bath clamp rods can be inserted. Don't loosen these screws too much or the captive nuts located within the translation stage will fall out. Ensure the bath clamp rods are firmly seated in the holes and then tighten the clamp screws. Give each clamp a slight tug to make sure that it is firmly fastened.

Loosen the tissue bath vertical translation stage clamp screw and slowly raise the bath upwards. It is convenient to hold onto one of the bath clamp rods to raise and lower the bath. Ensure that the lower tissue clamp and support rod enter the top of the bath without touching the glass bath. If there is interference then check that the bath clamp rods are in place properly. The bath clamp rods were cut to length so that when the rods are bottomed out in the holes the bath will be at the correct distance from the vertical plate. Ensure that you always support the vertical bath translation stage when moving it. Under no circumstances

should you allow the bath translation stage to fall unsupported. Doing this could break the bath. Tighten the bath vertical translation stage clamp screw when not moving the stage. **Caution:** when both the bath and the outer jacket are filled with liquid the bath and vertical translation stage will weigh about 3 kg. Ensure that you have a good grip on the bath vertical translation stage before loosening the stage clamp.



2.4 Attaching the Oxygenating Bubbler

The Radnoti tissue bath comes complete with an oxygenating bubbler attachment. Insert the bubbler in the second port up from the bottom of the tissue bath. Lock it in place by gently tightening the black lock screw. A tube-to-luer adapter fitting is provided to allow 1/8" ID Tygon tubing to be attached to the bubbler. The bubbler includes a flow control valve. Connect an oxygen supply to the bubbler then use the valve to regulate the flow of oxygen.

2.5 Connecting the Bath Drain

The Radnoti bath comes with a bath drain valve. Simply push the luer fitting on the drain valve into the outlet on the bottom of the bath. Tygon tubing can then be connected to the drain valve.

2.6 Attaching the Temperature Controller to the Tissue Bath

There are two ports on the Radnoti tissue bath for water to circulate through the outer jacket of the bath. Use 5/16" OD x 3/16" ID Tygon tubing to make the liquid connections. Attach the tubing to the Radnoti bath using the procedure shown on the Radnoti "Quick Disconnect Instructions" page located at the end of this manual. It is recommended to connect the outlet tube from the temperature control circulator to the bottom connection on the bath and the return line to the circulator to the top outlet. This ensures any air in the system will be returned to the circulator and not get trapped in the bath. If required the bath can be rotated within the band clamps by loosening the clamp screws on each bath clamp band and then repositioning the bath in the clamps as required.

A black plastic tube clamp is provided on the right side of the vertical mounting plate. Place the inlet and outlet hoses in the clamp and then locate the clamp at a convenient location next to the tissue bath. This clamp provides support and strain relief for the water circulation hoses.

2.7 Connecting a Stimulator to the 810A

CAUTION – Turn off the stimulator and unplug it from the BNC connector on the 810A before touching the electrodes. Care must be taken to not contact the stimulation electrodes whenever the stimulator is powered. The electrode holder, lower tissue clamp and the upper clamp holding the support tube are all made from Delrin and therefore are all insulated from the rest of the apparatus. Do not touch the lower clamp support tube while the stimulator is operating.

A BNC connector is located on the top right side of the 810A next to the tissue clamp vertical translation stage. Use a BNC to BNC patch cable to connect the output of the stimulator to this connector.

A red and black wire are connected to the BNC connector and run to two contacts located in the electrode mount. Four gold plated mating contacts (2 are required and 2 are provided as spares) have been supplied in the box with the motor mount screws and the Allen keys. The stimulation electrodes should be attached to these contacts and then the contacts can be plugged into the mating contact on the electrode holder. The electrodes can be soldered to the gold contacts however care should be taken to not get any solder on the

mating surface of the contact. If desired a length of stiff wire can be soldered to the contact and then the electrode soldered to the wire. This allows shorter electrodes to be used but ensure that the electrodes are positioned next to the muscle. Some researchers have discovered that the presence of solder in the bath can allow heavy metals (lead) to leach into the bath and affect the muscle. If extension wires are used then steps should be taken to prevent solder from coming in contact with the bath solution. Two methods to prevent contact are coating the solder connection with rubberized paint or covering the connection with melt heat shrink tubing. This type of heat shrink tubing contains a layer of material on the inner wall that melts and flows to produce a watertight seal inside the heat shrink.

3.0 Using the 810A

3.1 Adjusting the Upper and Lower Limits of the Bath Translation Stage

There is a right angle bracket located beneath the bath translation stage. This bracket can be repositioned to control the lower limit of motion of the bath translation stage. Simply loosen the screw, slide the bracket to the new position and retighten the screw.

Likewise there is an upper limit stop. This stop has a black plastic knob on it to allow the user to easily loosen the screw and slide the stop to a new position. Care should be taken to ensure that the stop prevents the bath from being raised too far and causing the lower tissue clamp to hit either the oxygenating bubbler tube or the bottom of the bath.

3.2 Adjusting the Lower Tissue Clamp Assembly

The vertical position of the Lower Tissue clamp assembly can be controlled in three ways. The first is a fine adjustment screw located above the tissue clamp assembly. Loosen the two locking knobs and then rotate the adjustment screw to raise or lower the tissue clamp assembly. Ensure that the locking knobs are retightened before proceeding to test a muscle. Since the 310B-LR muscle lever is capable of generating up to 100 N (10 kg) of force it is important that all locking knobs are tight before starting a test. The second method is a coarse adjustment that is made by relocating the tube clamp plate vertically on the side of the tissue clamp vertical adjustment stage. Simply loosen the screw holding the plate in place and then slide the plate to the new position before retightening the screws. The third method is to relocate the bracket that holds the fine adjustment screw. Loosen the clamps holding the fine adjustment stage then loosen the bracket and slide the entire lower tissue clamp/electrode clamp assembly up or down. This is the most difficult of the three adjustments and should only be used if you run out of vertical adjustment room using the other two methods. Ensure that you hold the entire assembly firmly when you loosen the bracket. If it slides down in an uncontrolled manner the lower tissue clamp can break the tissue bath.

3.3 Adjusting the Location of the Lever Arm

The location of the lever arm with respect to the lower tissue clamp and the centreline of the bath is controlled by the motor mounting screws. The motor mount plate has slotted holes in it to allow the motor to be adjusted in the horizontal direction to locate the lever arm above the centreline of the bath. Simply loosen the motor mount screws, slide the motor to the new position and retighten the screws.

3.4 Attaching Muscle to the 810A

CAUTION – to prevent electrical shock turn off the stimulator and unplug the BNC cable from the 810A before attaching the muscle tissue. The stimulation electrodes will be exposed during the process of attaching the muscle.

The muscle is located vertically between the lever arm and the lower tissue clamp. The muscle must be positioned so that it will be inside the bath when the bath is raised. Depending on the length of the muscle this normally means that the muscle should be clamp quite close to the lower tissue clamp. It is recommended that before attaching the muscle the vertical translation stage for the lower tissue clamp is adjusted to be close to its upper limit of

movement. Once the muscle is attached then there will be the full vertical range of movement available to set the resting length and tension of the muscle. It is also recommended that the Length Offset potentiometer knob on the front panel of the 310B be set to its middle position (5 on the turns counting dial).

Lower End of Muscle

Loosen the four screws on the front of the lower tissue clamp (do not loosen the screws that hold the clamp to the vertical stainless steel support tube). Position the muscle tissue (usually part of the tendon) between the plates of the clamp and tighten the screws. Alternatively you could suture to the muscle and then clamp the suture material in the lower tissue clamp. Another option is to hold a wire hook in the clamp and attach the muscle to the hook. Whatever method you use you must ensure that the tissue is held firmly by the lower tissue clamp and the material you use to hold the muscle doesn't stretch.

Upper End of Muscle

Suture material or wire can be attached to the upper end of the muscle (normally attach to the tendon) and then attached to the lever arm of the 310B. The 310B has a hole in the end of the lever arm that can be used for a hook or simply tie the suture through the hole. A small clamp can be fashioned and attached to the threaded hole on the lever arm but if this method is used the clamp must be made as small and light as possible. When attaching the upper end of the muscle an effort should be made to minimize any slack in the attachment since there is a limited range of vertical movement of the lower tissue clamp assembly.

3.5 Adjusting the Resting Tension

Ensure that the data acquisition program is running on the control computer before attaching muscle tissue. Likewise the 310B should be turned on and all controls adjusted correctly. This ensures that the arm will be held in the correct starting position and that force and length can be viewed on the computer screen.

Once the muscle is attached loosen the clamp screws on the fine adjustment vertical translation stage of the lower tissue clamp. Turn the fine adjustment knob while monitoring the force output on the computer screen. Once the desired resting tension value is reached lock the vertical translation stage in place. You will probably notice that the resting tension changes slightly when the stage is locked. Use the Length Offset potentiometer knob on the front panel of the 310B to move the arm up or down to obtain the desired resting tension. Normally you shouldn't need to move the Length Offset knob more than a few minor divisions either way to achieve the desired resting tension.

3.6 Measuring the Resting Length

Before raising the bath into place use a Vernier caliper or ruler to measure the resting length of the muscle tissue.